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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 9 JANUARY, 1951 No. 1 3

NEW YEAR'S GREETING

Food and nutrition continue to be of paramount importance, and it is encouraging that more people in our country and throughout the world are beginning to realize it. Signs of the time: United Nations Food and Agriculture Organization and World Health Organization increase nutritional activities in their programs; United States Public Health Service appoints (for the first time) medical nutrition consultants; American College of Surgeons in last annual meeting stresses importance of nutrition in pre- and postoperative care; recent research finding gives strong confirmation of the vital role diet has in prevention and treatment of "hardening of the arteries"; United States Civil Defense Handbook points out importance of nutrition in defense plans; subscriptions to *Nutrition Reviews* increase. Who can doubt the statement that nutrition is the single most important environmental factor affecting our health—whether we think of the health of a nation or the health of an individual!

This year begins the ninth year of *Nutrition Reviews*. Subscribers exist in every state of the Union and in nearly every country of the world, including Russia. The journal is translated into Portuguese and Spanish. Whatever good name the journal may have received for sound evaluation of current research in nutrition is the result of the sincere efforts of the Editorial Staff. Credit for getting copy in on time, for the detail of the index, and for the unusual accuracy of the journal rests squarely with the Assistant Editor. The Editor wishes to record his appreciation to the Assistant Editor and the Editorial Staff whose diligent efforts result in the publication of *Nutrition Reviews;* and last, but not least, to the Nutrition Foundation, Inc., for the complete editorial freedom enjoyed by the staff. We shall strive to make 1951 another successful year!

FREDRICK J. STARE, M.D.

NUTRITIONAL VALUE OF FROZEN FOODS

Freezing has become one of the important means of preservation of foods for relatively long storage periods. The use of frozen foods has increased greatly since their introduction for public approval, and gain in production and use of them continues. This can be illustrated by the production figures for frozen vegetables. The total American frozen vegetable pack from 1926 to 1930 amounted to (partially estimated) 100,000 pounds. The corresponding figures for 1947 were 347,663,000 pounds (*Western Canner and Packer* **41** (*No. 5*), *213* (*1949*)). Among the newer arrivals in the grocery store is frozen orange juice concentrate, production and sale of which has shown a phenomenal increase in a very short time. Thus in 1946, 2.48 million pounds were packed; by 1949 this had increased to 134.16 million pounds (*Ibid.* **42** (*No. 7*), *224* (*1950*)).

There seems to be almost no end to the variety of frozen foods that one can find on the market. These include not only fruits, vegetables, meats, poultry, and fish but also precooked foods and other specialty items. This wide popular acceptance was brought about by the appearance and taste appeal of these foods when placed on the consumer's table, as well as the time-saving features involved in use of them. Because of this in-

creased consumption, knowledge of the nutritional value of these foods has become increasingly important. Foods preserved by freezing must be nutritious if this form of storage is to yield products that will help to supply the dietary requirements of man.

Since chemical changes are known to take place more slowly at lower temperatures, frozen foods, if properly processed and stored, should undergo smaller losses of vitamin content than those preserved by methods which involve processing and storage at higher temperatures. Other changes, many perhaps only indirectly related to nutrition, occur to a lesser extent during storage of frozen foods. In the packing of fruits and vegetables several considerations are necessary to obtain a high quality finished product:

(1) Selection of variety is important. (2) The produce should be harvested in prime condition. In this stage, higher quality products will be obtained as far as appearance and flavor are concerned. (3) Proper blanching, in the case of vegetables, is necessary to inactivate enzymes. This prevents the development of undesirable off-flavors and loss of nutritive value during storage. (4) Rapidity of handling in the processing plant after harvesting is essential so that a minimum of time is allowed to elapse between the time of harvest and the entrance of the packaged product into the freezer. If this is not done, considerable loss of quality can result at this stage. (5) Storage at 0°F. or lower is necessary.

Vitamins and other nutrients as related to processing and storage: Considerable work has been done on the vitamin content of frozen fruits and vegetables, especially the content of vitamin C. This vitamin occurs in these two groups of foods frequently in quantities large enough to be of considerable significance. To produce frozen fruits and vegetables that are high in nutritional factors, careful attention to the foregoing five points should be given.

Research work has indicated that while nutritive values may vary from year to year depending on such variable factors as climate, soil, and sunlight, different varieties of any fruit or vegetable may vary widely in nutritive value. In this case, selection of the variety that is highest in vitamin content but still has high quality in so far as color, texture, and flavor are concerned, is desirable.

Fruits and vegetables harvested in the prime of condition not only will yield higher quality products as far as appearance and flavor are concerned, but frequently the vitamin content is at or near the peak at this stage.

TABLE I

Vitamin C Content of Peas (Telephone Variety) During Preparation for Freezing (Fresh Weight Basis)

Treatment	Ascorbic acid content (mg. per 100 g.)
Freshly vined peas	23
After washing	23
After blanching	21
Before quality separator	21
After quality separator	18
Before picking belt	16
After picking belt	16
After packing	16

Adapted from: R. R. Jenkins, D. K. Tressler, and G. A. Fitzgerald (*Food Res.* **3**, *133* (*1938*)).

Proper blanching not only prevents development of undesirable off-flavors, but it retards the storage loss of carotene and ascorbic acid. Blanching in water frequently results in a loss of ascorbic acid up to 16 per cent (C. G. King and D. K. Tressler, *Proc. Food Conf. Inst. Food Technologists, p. 123* (*1940*)). The over-all processing loss of this vitamin, in the case of peas, is approximately 30 per cent, as illustrated by the figures in tables I and II.

More water-soluble materials are lost during water blanching if a temperature lower than that of boiling water, coupled with a longer blanching time, is used to inactivate the enzymes. The minimum amount

of loss was found when boiling water was used, due to the shorter time necessary to inactivate the enzymes under these conditions. Since many of the vitamins, as well as the sugars and other substances found in the vegetables, are soluble in water, it is obvious that vegetables intended for freezing preservation should be blanched at the higher temperature and at the shorter time to avoid these losses. Losses of soluble materials tend to be smaller when blanching is done in flowing steam (N. B. Guerrant *et al.*, *Ind. Eng. Chem.* **39,** *1000 (1947)*).

Rate of freezing has been given considerable attention. While much has been said concerning the necessity for quick freezing

TABLE II

Ascorbic Acid Content of Fresh, Frozen, and Thawed Peas (Thomas Laxton Variety)

Material	Cooking time (min.)	Ascorbic acid retained in drained peas (mg. per 100 g.)
Fresh peas.....	0	23
Fresh peas.....	16	11
Frozen peas.....	0	17
Frozen peas.....	8	12
Thawed peas....	0	17
Thawed peas....	8	12

Adapted from: F. Fenton and D. K. Tressler (*J. Home Econ.* **30,** *717 (1938)*).

(where the center of the package reaches 0°F. in one and a half to two hours), recent work has demonstrated that equivalent quality and equally satisfactory retention of the vitamin content results when the time required to reach 0°F. is lengthened to eight to twelve hours (F. A. Lee, W. A. Gortner, and J. Whitcombe, *Ind. Eng. Chem.* **38,** *341 (1946)*; Lee and Gortner, *Refrig. Eng.* **57,** *148, 184 (1949)*; Lee, Gortner, and Whitcombe, *Food Technol.* **3,** *164 (1949)*; Lee *et al.*, *Food Res.* **15,** *8 (1950)*). This means that products frozen and stored in home-freezing cabinets can be as nutritious and palatable as those obtained by "quick-freezing." These facts were demonstrated with fruits, vegetables, and beef. However, one could not recommend extremely slow freezing, such as would require two days to two weeks to reach a center temperature of 0°F., because of other changes that might result.

I. A. Smorodintsev (*J. Applied Chem. (U.S.S.R.)* **16** *(Nos. 11–12), 368 (1943)*; *Chem. Abst.* **38,** *6410 (1944)*) found that meat proteins are not cleaved or denatured by the freezing process. J. M. Ramsbottom and C. H. Koonz (*Food Res.* **4,** *425 (1939)*) found that regardless of the rate of freezing, the histologic appearance of defrosted muscle tissue is similar to that of unfrozen muscle tissue.

TABLE III

Analyses of Tendergreen Snap Beans After Different Speeds of Freezing (Wet Weight Basis)

Sample	Ascorbic acid (mg. per 100 g.)			Carotene (micrograms per 100 g.)			Thiamine (micrograms per 100 g.)		
	A	B	C	A	B	C	A	B	C
Raw.....	23			380			96		
Blanched.....	18			360			93		
−40°F. airblast.		17	12		328	400		81	73
0°F. still air....		18	12		337	368		87	69

(A = raw and blanched; B = analyzed immediately after freezing; C = analyzed after storage for six months at −6°F.)

Adapted from: F. A. Lee, W. A. Gortner, and J. Whitcombe (*Ind. Eng. Chem.* **38,** *341 (1946)*).

Consideration of suitable storage conditions for frozen foods is particularly important, not only for fruits and vegetables, but for all classes of frozen foods. Storage at 0°F. or lower is necessary for satisfactory preservation for relatively long-term storage. It has been shown that frozen foods stored at temperatures higher than this show color deterioration and losses in ascorbic acid. It is obvious that storage temperatures higher than 0°F. materially shorten the storage life of these foods. While it has been shown that storage at temperatures somewhat higher than 0°F. shortens their storage life, W. A. Gortner, F. Fenton, F. E. Volz, and E.

Gleim (*Ind. Eng. Chem.* **40**, *1423 (1948)*) found that constant temperature of storage is not absolutely necessary provided that this storage temperature does not rise above zero. The average storage temperature is the more important factor. It follows that one is safe in stating that fluctuating storage temperatures below 0°F. have little effect on the food, at least when stored for reasonable periods of time.

Frozen pork roasts, strawberries, snap beans, and peas were stored for twelve months at 0°F. and 20°F. in a repeating six-day cycle. Fat rancidity was evident after four months at temperatures above zero, but did not occur after one year at 0°F. Ascorbic acid losses during storage at 10°F., or at the fluctuating temperatures, ranged from approximately 50 per cent of the total of ascorbic acid in peas to 65 per cent of that present in strawberries and 80 per cent of that in snap beans during a storage period of one year. At 0°F. all this vitamin was found to be retained in peas, and only one-third was lost from the other products during one year of storage. Palatability changes, in general, paralleled these chemical changes. The thiamine content of pork was not affected by the storage temperatures or the storage period. Exposure of frozen food to temperatures fluctuating between 0°F. and 20°F. resulted in quality changes similar to those occurring in food stored at 10°F. The quality of the food stored under both of these conditions was definitely inferior to that stored at 0°F. It seems, therefore, that exposure of frozen foods to temperatures above 0°F., rather than merely temperature fluctuation, may be a major factor influencing deterioration in food quality.

Fortification with ascorbic acid: D. K. Tressler and C. Du Bois (*Food Industries* **16** (*No. 9*), *75, 137 (1944)*) found that ascorbic acid, when added to the syrup used to cover peaches prior to freezing, controlled the browning which resulted when the product was defrosted. At the present time, about 150 to 200 mg. of ascorbic acid are added to 1 pint of finished product. While this is added to control browning, as an improvement in the freezing of peaches, it has the added advantage of increasing the nutritional value of the product. This process is also of value in the freezing of sweet cherries.

F. A. Lee, H. G. Beattie, W. B. Robinson, and C. S. Pederson (*Fruit Prod. J.* **26**, *324 (1947)*) found that by fortifying freshly pressed apple juice with 40 mg. of ascorbic acid per 100 ml. of juice, the product could be frozen and stored at 0°F. without development of a musty flavor. At the same time, the very light color of the freshly pressed juice was preserved. Again, the nutritional value of the product was increased.

H. L. A. Tarr (*J. Fisheries Res. Board of Canada* **7** (*No. 3*), *137 (1947)*) proposed the use of an ascorbic acid-dip to inhibit rancidity in frozen fish fillets. J. C. Bauernfeind, E. G. Smith, O. M. Batcher, and G. F. Siemers (*Quick Frozen Foods* **10** (*No. 8*), *139 (1948)*; **10** (*No. 9*), *68, 72 (1948)*) found that by dipping the fillets in an aqueous solution or light brine solution of ascorbic acid, better retention of quality resulted in the frozen fish.

Frozen foods are nutritious, palatable, and pleasing to the eye, and occupy an important place in the American diet.

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REVERSIBILITY OF THE EFFECTS OF UNDERNUTRITION IN CHILDREN

Five years after the end of World War II it begins to be possible to assess at least some of the effects of prolonged (wartime) undernutrition in children and of the subsequent measures taken by individuals and governments to remedy this condition. Mass-

scale examinations conducted in Great Britain, Holland, and Switzerland during the war and immediately afterwards have already been published. For instance, in Britain E. R. Bransby (*Monthly Bull. Ministry Health* **3,** *212* (*1944*)) had concluded that there was no delay in the heights and weights of British schoolchildren between 1940 and 1943. The only observed abnormality was that children living in country groups exhibited a slower rate of growth than children living with their families, despite an adequate diet. The same author concluded later (*Med. Officer* **73,** *165* (*1945*)) that a height-weight relationship gave a better correlation with the general state of nutrition (as estimated by clinical examination) than did the standard weight curve. E. H. H. Milligan and E. L. Fanning (*Ibid.* **67,** *77* (*1942*)) had, during the same period, observed a slight delay in the heights of schoolchildren until 1941, this delay being corrected by 1944.

Similar studies conducted in Holland did not reveal any serious delay in the growth rate of children until 1944 (M. J. L. Dols, "Enseignements de la Guerre 1939–1945 dans le domaine de la Nutrition," *Masson et Cie, Paris* (*1947*)). In Switzerland, despite a decrease in the total food supply, it was possible, thanks to an improved distribution, to demonstrate an increase in the weights and heights of schoolchildren during the war period (A. Fleisch, *Ibid.*).

The most thorough and extensive surveys during the war and postwar period have been conducted in France, where the National Institute of Hygiene has been able to follow 800,000 children. The results of the statistical analysis of these data have just been published (J. Tremolieres *et al.*, *Rec. d. trav. Inst. nat. d'hyg.* **4,** *1* (*1950*)). The children, originating from various regions of France, were followed during the years 1940 to 1948. The age range was 6 to 20 years. It may be recalled that nutritional conditions in France during the war period were considerably worse than either in Britain or in Switzerland, though generalized starvation did not reach the desperate stage found in Holland in 1944. The two main conclusions of the report were the following:

First, the delay in growth of children was, as would be expected, most prevalent during the period of most acute food shortage (average intake 1600 to 2000 calories in cities). The maximum delay observed was in the group of 13-year-old girls where it reached 7 cm.

Second, this delay in growth appeared completely reversible. By the time these same children had reached the age of 16, the deficit had been eliminated. For all age groups the average heights in 1948 were equal or superior to the heights of corresponding groups in the prewar period.

Other significant points were brought to light by the statistical analysis of data pertaining to such large groups. In particular, it was shown that factors other than nutritional seemed to be correlated with size: in particular the type of training undergone by older children and adolescents; the number of brothers and sisters in the family (gradually a statistical decrease of up to 3 cm. is observed in large families); finally, the statistics show that city children are larger than country children. The authors emphasize that these factors, which they aptly call "microclimatic," must be taken into consideration when attempting to interpret statistics on heights and weights of children. This is made particularly striking by the fact that while the large, expected deviations from standards shown by children who had been undernourished for several years were relatively quickly eliminated, the small "microclimatic" differences proved to be much more stable even though in many cases the large temporary deviations had reversed the normal picture. Thus in the case of country and city children, although the former had fared better during the war period, by 1948 the latter exhibited again a small but significant superiority in size.

MOLYBDENUM IRON IN ANEMIAS OF PREGNANCY

Several of the trace minerals have been shown to be important in the prevention and cure of anemias induced experimentally or occurring naturally in animals (*Nutrition Reviews* **4,** *227, 291 (1946)*). The role of iron is well known. Copper is also essential under certain conditions. It is now known that cobalt is essential for hemoglobin formation since it is an apparently essential part of the vitamin B_{12} molecule, although a cobalt deficiency as such does not seem to be an etiologic factor in conditions which respond to vitamin B_{12}. Cattle in certain parts of Australia develop an anemia which responds to minute amounts of cobalt.

In the treatment of human anemias only iron has been convincingly demonstrated to be essential. Some favorable results with cobalt in anemias in children have been reported, but these results have not been confirmed (*Nutrition Reviews* **4,** *227 (1946)*). Copper is added to many of the iron preparations used in human anemias, but proof that it is of benefit is lacking. Most authorities seem to agree that copper is not essential in the treatment of any known anemia in adults although it may be of some benefit in nutritional anemias in children (W. J. Darby, *J. Am. Med. Assn.* **142,** *1288 (1950)*; *Nutrition Reviews* **4,** *291 (1946)*). The effectiveness of iron salts alone in most of the common secondary anemias is well known. However, the response to iron is often slow and in many instances incomplete. This has led to a continuing search for agents or methods which will improve the effectiveness of iron.

E. R. Neary (*Am. J. Med. Sci.* **212,** *76 (1946)*) explored the effectiveness of molybdenum when given in conjunction with ferrous sulfate. His subjects were pregnant women suffering with "true iron deficiency anemia of pregnancy." Pregnant women normally develop a moderate anemia, the so-called physiologic anemia of pregnancy, due to an increase in body water and blood volume. This anemia does not respond to iron since it is really a hydremia rather than an anemia. The patients studied by Neary, however, had blood values below the normal pregnancy standards. Of the 22 patients in his group, all had hemoglobin values of less than 10 g. per cent, 21 had erythrocyte counts of less than 3.36 million, and all had subnormal mean corpuscular hemoglobin concentrations.

The patients were divided into two groups of 11 each. One group received ferrous sulfate alone and the other received molybdenized ferrous sulfate. During treatment periods of six to seven weeks the group receiving the molybdenized ferrous sulfate gained 0.74 g. per cent hemoglobin per week, reaching an average value of 13.5 g. per cent, while those receiving iron alone gained only 0.27 g. per cent per week, attaining an average final value of 11.1 g. per cent. After the group receiving iron alone had reached a plateau in their hemoglobin response they were given molybdenized iron. Each patient showed a rapid response reaching virtually normal values. The lack of a complete response to ferrous sulfate alone was at least in part due to inadequate dosage. These patients received 240 mg. of ferrous iron daily, an amount which would be expected to be fully adequate, yet when this dosage was increased 50 per cent, a renewed hemoglobin response occurred. The molybdenized ferrous sulfate was given in an amount which also supplied approximately 240 mg. of ferrous iron plus 15 mg. of molybdenum, and this was fully effective. Neary believes that this indicates a greater efficiency of utilization of the molybdenized iron.

The molybdenized ferrous sulfate used in this study was prepared by a special process which involved co-precipitation of molybdenum sesquioxide and ferrous sulfate with the production of a complex in which elemental molybdenum and ferrous iron were present in an approximate ratio of 1:15.

This type of iron preparation has been

used by others. R. F. Chesley and J. E. Annito (*Bull. Margaret Hague Maternity Hosp.* **1,** *68 (1948)*) found significant increases in hemoglobin concentration in pregnant women given this special iron salt. More recently, a group of investigators at the University of Chicago and the Chicago Lying-in Hospital have used this product in the anemias of pregnancy. Their first report covers the effectiveness of molybdenized iron in treating pregnant women who were already anemic (W. J. Dieckmann and H. D. Priddle, *Am. J. Obstet. Gynec.* **57,** *541 (1949)*), while the second report deals with its use as a routine prophylactic measure (Dieckmann, Priddle, J. A. King, and J. B. Forman, *Ibid.* **59,** *442 (1950)*).

In the first study, 88 pregnant women with anemia were studied; 49 received molybdenized iron (6 capsules daily in three doses providing 1.17 g. of ferrous sulfate and 18 mg. of molybdenum sesquioxide daily), and 39 served as controls. The method for selecting the control group and what treatment they received, if any, is not stated. Apparently they did not receive iron in any form. Of the 49 treated patients, 45 gained an average of 2.11 g. of hemoglobin before delivery, having an average hemoglobin concentration of 11.8 g. per cent at this time. Three patients failed to respond and one could not tolerate the medicine. Twenty-three of those patients who were treated five to six weeks gained 2.36 g. per cent of hemoglobin or about 0.43 g. per cent per week. This is considerably less than that reported by Neary. The group used as controls showed a hemoglobin concentration of about 10.3 g. per cent at the start of the experimental period and were about the same (10.7 g.) at the time of delivery. The authors state that they "have never had other iron salts so efficacious in pregnant patients." Their results were "so striking" that if the patient had taken the medication for three weeks and showed no response, therapy was stopped and a more thorough examination was instituted to determine the cause of the anemia.

In their second study, the molybdenum-iron complex was given to an unselected group of outpatients beginning at about the middle of pregnancy and continuing to term. The patients were taken at random without knowledge of their blood picture. Fifty-six patients were used as untreated controls and 67 were given the special iron preparation. The hemoglobin and hematocrit levels of the two groups were almost identical until late in pregnancy. In the last ten weeks the group receiving the iron preparation increased their hemoglobin concentration more, so that at delivery their average value was 13.1 g. per cent as compared to 12.2 g. per cent in the controls. Means for the hematocrit in the treated and control groups were 39.5 and 38.1 volumes per cent, respectively. These results were considered as indicating a "much higher" hemoglobin concentration in the treated group, although a statistical analysis of neither the hemoglobin concentration nor the hematocrit differences was reported.

The studies of Neary and those of the Chicago group reemphasize a fact already well known, that some pregnant women develop an anemia which can be improved with iron therapy. They also show clearly that molybdenized ferrous sulfate is a safe and effective preparation to use in such patients. However, these studies fail completely to provide critical evidence that molybdenized ferrous sulfate will accomplish anything which adequate dosage with ordinary ferrous sulfate will not accomplish. Perhaps some weight should be given to the clinical impression of a group as experienced as the Chicago group when they state that they "have never had other iron salts so efficacious in pregnant patients." Nevertheless it seems difficult to justify any statement or inference that molybdenized ferrous sulfate is superior to ordinary ferrous sulfate preparations until more convincing evidence

from properly controlled experiments is forthcoming.

There is little reason from the experimental standpoint to suspect that dietary supplements of molybdenum might be essential in hemoglobin regeneration. This trace mineral is a normal constituent of almost all plant and animal tissues. There is some experimental evidence indicating that it plays a role in plant metabolism but none that it has any special function in animals. In view of the widespread occurrence of molybdenum and the minute concentration in animal tissues, it seems unlikely that the human diet would be deficient in this element.

Molybdenum in excess is known to cause scouring and other toxicity in cattle (W. R. Muir, *Vet. J.* **97,** *387* (*1941*); W. S. Ferguson, A. H. Lewis, and S. J. Watson, *J. Agr. Sci.* **33,** *44* (*1943*)). The herbage of pastures in certain areas of England and the United States contains a large amount of molybdenum. Cattle, grazing on this pasture, develop diarrhea and loss of weight which can be cured with copper salts. Although the mechanism of this action of copper is not known, it is possible that there is some interaction between copper and molybdenum which, conceivably, could effect blood formation, but this remains to be demonstrated.

NUTRITION OF HOST AND PARASITE

An important area of research in nutrition that promises to be of considerable practical importance is the differential influence of diet upon growth and physiologic well-being of parasite and host. Differences may be due to specific differences in requirement for dietary essentials or to unlike availability of nutrients; and the very improvement in metabolism in the host under one favorable nutritional adjustment may result in physiologic inhibition in the parasite and vice versa. A recent study of the survival and growth of the nematode, *Ascaridia galli*, in chicks fed natural and purified diets (E. H. Sadun, C. K. Keith, M. J. Pankey, and J. R. Totter, *Am. J. Hyg.* **51,** *274* (*1950*)) calls attention again to the general problem, with new bases for comparison.

As early as 1926 N. B. Zimmerman, L. B. Vincent, and J. E. Ackert (*J. Parasit.* **12,** *164* (*1926*)) observed that chickens provided with the vitamin B-complex are more resistant to infection with *Ascaridia galli* than when this dietary essential is missing from the diet. However, when yeast was present in the feed, the worms were longer than in its absence. Other vitamins were studied in this connection and it was found that with a deficiency of vitamin A, more and larger worms (*Ascaridia lineata*) were found than in the controls (Ackert, M. F. McIlvaine, and N. Z. Crawford, *Am. J. Hyg.* **13,** *320* (*1931*)). Although vitamin D deficiency did not appear to lower the resistance of the host to infection, it did appear to protect the host against the effects of *Ascaridia lineata* (Ackert and L. A. Spindler, *Ibid.* **9,** *292* (*1929*)). Other types of rations such as vegetables or meat or milk diets have been studied in this connection but improvements both in the purification of experimental diets and in the technic of feeding chicks with this type of ration justified the newer studies.

One-day-old single comb White Leghorn chicks were given the experimental diet before they had the opportunity to eat any other kind of food. When the chicks were 9 days old they were infected with five hundred embryonated eggs of *Ascaridia galli*, and at 32 days of age they were killed. The intestinal worms were counted and measured, the number recovered in relation to the egg dose being used as the criterion for estimating the degree of resistance of the

chicks to the parasites. In addition the liver, spleen, and thymus were weighed and total erythrocyte, total leucocyte, and differential white cell counts were made; thus the physiologic state of the host was judged.

Inasmuch as folacin is one of the newer members of the vitamin B-complex and is a requirement for the chick (A. G. Hogan and E. M. Parrott, *J. Biol. Chem.* **128,** *xlvi* (*1939*)), this factor was used as the variable in the purified diets. Four experimental rations were used: (I) a basal diet of purified constituents, deficient in folacin; (II) the same ration plus 15 micrograms of folacin per 100 g. of diet; (III) the basal ration plus 200 micrograms per 100 g. of diet; (IV) a commercial starter food. Fifteen micrograms of folacin per 100 g. of diet are insufficient for normal development of the chick, whereas 200 micrograms are more than the minimum requirement of this factor (C. K. Keith *et al.*, *Ibid.* **176,** *1095* (*1948*)).

The difference in the rate of growth of the chicks was apparent in seven days; at 30 days of age groups III and IV averaged 262 and 244 g., respectively; group II, 179 g.; and the negative control group I, 120 g. The livers of group I were greatly enlarged weighing almost twice that of the positive control groups III and IV. The spleens of group I were somewhat reduced in size and the thymus glands of this group were greatly reduced (0.08 g.) compared to those of group III (0.48 g.). The blood studies showed anemia and leukopenia, with a reduction in the number of lymphocytes and basophilic leucocytes in the folacin-deficient chicks.

The number of worms in the intestine of the chicks were counted twenty-three days after infestation and there is apparent a striking correlation with the nutritional adequacy of the diet to which the chicks were restricted. The negative control group (I) showed an average of 17.3 worms; group II, 9.8; groups III and IV, 4.9 and 6.9, respectively. The leukopenia and the atrophy of the thymus which were demonstrated in the folacin-deficient group (I) may indicate a reduced degree of resistance through the reduced activity in antibody formation grossly reflected in the relatively greater number of nematodes harbored by the chicks of this group. When the length of the worms was measured, it was found that of the groups on the purified rations, group I had the longest worms. However, in the chick fed the commercial starter ration, the worms were five times longer than those in group I. The reason for this latter observation is not clear. On the purified rations, however, there is an inverse relation between the length of the worms and the amount of folacin in the diet.

Inasmuch as the purified diets contained no vitamin B_{12}, 2 per cent dried liver was added to the purified basal ration for one group of chicks infected with *Ascaridia galli* and 8 ml. of 15-unit injectable liver extract per kilogram of body weight to a second group of chicks. The chicks grew better on the diets containing liver than on the commercial starter ration. The group consuming the diet with dried liver had fewer and shorter worms than did the group with the injectable liver extract.

The results of the present study throw new light on the problem of host-parasite nutritional relations. It appears, in general, that a specific dietary lack—in this case folacin deficiency—affects in an adverse way the ability of chicks to resist infection with *Ascaridia galli*. It has similarly been shown (P. L. Day, W. C. Langston, and C. F. Shukers, *J. Nutrition* **9,** *637* (*1935*); M. Janota and G. M. Dack, *J. Infect. Dis.* **65,** *219* (*1939*)) that in monkeys on a folacin-deficient ration, dysentery develops with presumably a latent infection becoming acute as the host loses the power of resistance.

THIAMINE-SPARING ACTION OF CERTAIN SULFONAMIDES

A variety of nutritional deficiencies may evolve in animals fed purified diets containing sulfonamides. These are shown to result from inhibition of synthesis of essential nutrients by organisms residing in the intestinal tract and are corrected by addition to the diet of specific food factors. In other instances deficiencies have been prevented or corrected by adding para-aminobenzoic acid, an antagonist, to diets containing sulfonamides (*Nutrition Reviews* **2,** *221* (*1944*); **3,** *48* (*1948*); **8,** *260, 331* (*1950*).

In 1946 E. C. Slater of the Australian Institute of Anatomy in Canberra (*Nature* **157,** *803* (*1946*)) reported preliminary data indicating that one of the group of sulfonamide drugs, sulfadiazine, directly influenced utilization and metabolism of thiamine in the human being and the rat, an effect which was apparently not related to inhibition of bacterial synthesis of the vitamin. These investigations have been extended and are described in detail in a recent paper (C. C. Kratzing and Slater, *Biochem. J.* **47,** *24* (*1950*)). The experimental data indicate that sulfadiazine and sulfamerazine have a sparing action on thiamine in the body of the rat and that this effect, unlike that of sulfonamides on bacterial metabolism, is not inhibited by para-aminobenzoic acid.

Animals used were hooded rats which were individually housed and were 4 to 5 weeks of age at the start of the experiment. The food consumption of each rat was adjusted so that rats from the same litter but in groups fed different experimental diets were permitted to grow at a rate equal to that of the slowest growing member of the litter.

In earlier experiments a diet was fed which was deficient in para-aminobenzoic acid, folacin, and biotin. This was necessitated in the attempt to determine whether the effect of sulfonamides on animal tissues was reversed by para-aminobenzoic acid. At the time the studies were initiated preparations of folacin and biotin were not available in sufficient quantity to prepare a purified diet which was essentially free of para-aminobenzoic acid but contained these two vitamins in adequate quantities. Having established that para-aminobenzoic acid did not reverse the sulfonamide effect it was possible later to use a diet containing para-aminobenzoic acid, folacin, and biotin. The thiamine content of the diets was of the order of 15 micrograms per 100 g. dry weight of diet. Supplements of this vitamin were fed daily to each rat by pipet. All other vitamins were incorporated in adequate quantities in the diet. Sulfonamides were included in the food.

At the end of each experiment animals were killed and the weights of the liver, kidneys, and thyroid gland determined. Analyses were made of the thiamine contained in the liver and in the carcass excluding the liver. A modification of the thiochrome method was utilized and is described in detail in the paper. It was shown that there was a direct relationship between thiamine intake and content of the vitamin in liver over a range of daily dietary intake of 7.6 to 20.7 micrograms and that an intake of 20.7 micrograms per day is not adequate to produce tissue saturation in young rats. In addition to thiamine analyses the concentration of pyruvate and of sulfonamide in blood was determined in certain experiments at the time the animals were killed.

In a first experiment rats were fed the diet deficient in para-aminobenzoic acid, folacin, and biotin with the intake of thiamine adjusted to 6.9 micrograms per day. Sulfadiazine was added to the diet in a concentration of 0.55 per cent of the dry weight. A control group of rats received an identical diet without sulfadiazine. At the end of the experiment after thirty-two days on the diet the total thiamine contained in the livers of animals receiving sulfadiazine averaged 16.6 micrograms, whereas this

value in the control animals was only 9.8 micrograms. The weight of livers was increased 10 per cent and the weight of kidney decreased 11 per cent in animals getting the drug. These differences are shown by statistical methods to be significant.

The diet given provided an intake of 10.3 micrograms of riboflavin per day. Analysis of the liver content of riboflavin showed no change occurring with inclusion of sulfadiazine in the diet of the rats. This seemed to indicate that the sparing action of sulfadiazine on thiamine metabolism had no counterpart in riboflavin economy.

In another group of rats receiving the same diet but succinylsulfathiazole rather than sulfadiazine there was no change in concentration of thiamine in the liver nor in the weight of the liver or kidney. Succinylsulfathiazole in contrast to sulfadiazine is poorly absorbed from the intestinal tract and its anticipated effect on the bacterial flow would be greater. It seemed unlikely, therefore, that the thiamine-sparing effect was mediated through changes in bacterial flora in the intestine and was more likely a direct effect on animal tissues.

In further experiments with rats given this diet containing either equimolecular quantities of sulfadiazine and para-aminobenzoic acid or the same quantity of the antimetabolite without the sulfonamide, it was found that para-aminobenzoic acid, alone, had no effect on liver thiamine and did not reverse the effect of sulfadiazine. It was shown, also, that thiamine-sparing or increase in tissue thiamine occurring with feeding of sulfadiazine was not limited to the liver, since a similar increase was demonstrated in the rat carcass from which the liver had been removed.

The effect of sulfadiazine in sparing tissue thiamine was shown as well when the diet was changed to include adequate quantities of para-aminobenzoic acid, riboflavin, folacin, and biotin. Furthermore, the percentage increase in content of thiamine in the liver was nearly the same in animals receiving 20.7 micrograms thiamine per day, a much higher but perhaps still not adequate level of intake of the vitamin.

In a search for added evidence of protection of thiamine stores, the concentration of pyruvate in blood was determined with rats in a resting phase at the time they were killed. There is impairment in pyruvate metabolism with an increase in concentrations of pyruvate in blood and tissues in thiamine deficiency. In animals receiving a low level of intake, 7.6 micrograms of thiamine per day, the concentration of blood pyruvate on the twenty-first day was increased with a mean of 2.4 mg. per 100 ml. When 0.55 per cent sulfadiazine was provided in the diet, the mean blood pyruvate was 1.3 mg. per 100 ml., a value which is less by a significant degree. When higher blood levels of the sulfonamide were achieved by parenteral injection of solutions of the drug there was further reduction in concentrations of blood pyruvate. In all groups of animals there was a significant negative correlation between levels of pyruvate in blood and thiamine content of the liver.

As further evidence of the thiamine-sparing action of sulfadiazine, modification of the manifestations of thiamine deficiency was observed when the drug was added to the thiamine-deficient diet. Unlike control animals which did not receive sulfadiazine, typical signs of the deficiency did not appear and the body weight lost was much less. However, the drug was not completely protective. Animals finally died in convulsive seizures.

The effect of sulfadiazine was compared with that produced by the feeding of other members of the group of sulfonamide drugs. Of those fed, only sulfamerazine, which is similar in structure to sulfadiazine, was found to have a comparable effect in the sparing of thiamine. Sulfanilamide, sulfathiazole, and sulfapyridine as well as succinylsulfathiazole, which was tested in other experiments, were almost wholly without effect.

It was of interest that in the experiments of Kratzing and Slater (*loc. cit.*) sulfadiazine and sulfamerazine were the only sulfonamide drugs which produced thyroid enlargement. Other investigators have described this effect of the sulfonamides (C. G. MacKenzie and J. B. MacKenzie, *Endocrinology* **32,** *185 (1943)*; E. B. Astwood, J. Sullivan, A. Bissell, and R. Tyslowitz, *Ibid.* **32,** *210 (1943)*). The association of thyroid hypertrophy with the thiamine-sparing action of sulfadiazine and sulfamerazine suggests that the effect of these drugs on thiamine metabolism might result from a decrease in the basal metabolic rate. It is known that the thyroid hyperplasia produced by sulfonamides results in substantial reduction in basal metabolic rates. It would be of real interest to attempt substantiation of this explanation of the thiamine-sparing effect by evaluating the effect of unrelated substances such as thiourea, which also are effective in decreasing synthesis of thyroxine.

There is little to support any other mechanism of action which would explain thiamine-sparing by sulfadiazine and sulfamerazine. The authors consider the possible roles of increased renal threshold for thiamine, bacterial synthesis of the vitamin, acetylation of sulfonamides, and inhibition by the drugs of enzymes containing thiamine in a prosthetic group. Neither in their data nor in the experiences of other investigators can they find reasonable support for these modes of action.

These experiments provide important evidence that sulfadiazine and sulfamerazine have a direct action on tissues which protects and spares the thiamine available when animals are on low levels of intake. The effect is one which must be considered in experiments which utilize these sulfonamides in studies of bacterial synthesis. If it can be shown conclusively that thiamine sparing results from lowered metabolic requirements, it would seem that the effect might involve other essential nutrients as well as thiamine.

REVERSAL OF AMINOPTERIN INHIBITION

Aminopterin (4-aminopteroylglutamic acid; 4-aminofolic acid) is an analogue of folacin in which the hydroxyl group of the pteridine ring of the vitamin has been replaced by an amino group. Originally synthesized by D. R. Seeger, J. M. Smith, and M. E. Hultquist (*J. Am. Chem. Soc.* **69,** *2567 (1947)*), this compound has been tested in a variety of species as an antagonist of folacin, and has proved to be an exceptionally potent antivitamin. At low levels of administration, in many organisms, its toxic effects can be prevented by simultaneous administration of folacin, but at higher levels of administration, even large amounts of folacin do not prevent its toxic effects (*Nutrition Reviews* **6,** *338 (1948)*; **7,** *310 (1949)*; **8,** *260 (1950)*; A. L. Franklin, E. L. R. Stokstad, and T. H. Jukes, *Proc. Soc. Exp. Biol. Med.* **67,** *398 (1948)*). Thus, although the compound appears to act by inducing a deficiency in folacin or its enzymatically active derivatives, the latter vitamin does not always fully counteract its effects, *i.e.*, the antagonistic relationship is not fully competitive.

If inhibition by aminopterin results from inhibition in the organism of reactions normally catalyzed by folacin or its derivatives, then this inhibition should be alleviated by adding to the nutrient environment the products of the inhibited reactions. From studies with microorganisms, it appears that thymine and purine bases or related products are among these products, since a mixture of these substances eliminates the nutritive requirement of certain lactic acid bacteria for this vitamin, and also counteracts the inhibitory action of methylfolic acid for the same organisms (*Nutrition Re-*

views **7,** *49* (*1949*)). Furthermore, it is known that thymidine (but not thymine) counteracts the inhibitory action of aminopterin for certain strains of *Escherichia coli* (A. L. Franklin *et al., J. Am. Chem. Soc.* **71,** *3549* (*1949*)) and *Leuconostoc citrovorum* (H. E. Sauberlich, *Arch. Biochem.* **24,** *224* (*1949*); *Nutrition Reviews* **8,** *282* (*1950*)). It has not been known whether similar systems were involved in animal metabolism.

A study by E. E. Snell and W. W. Cravens (*Proc. Soc. Exp. Biol. Med.* **74,** *87* (*1950*)) throws some light on this question. These workers found that embryonic development of hen eggs was inhibited completely if 100 micrograms or more of aminopterin were injected per egg just prior to incubation. Such eggs could not be differentiated from infertile eggs by gross examination. Fifty micrograms or less of aminopterin permitted initiation of development in some eggs; the number showing any development, and the period of embryonic development before death increased as the amount of aminopterin decreased. Five micrograms was the highest amount of the drug that permitted any live chicks to hatch; with this amount of the drug 23 per cent hatch (as compared with 70 per cent in water-injected controls) was obtained.

The effectiveness of various compounds in counteracting aminopterin inhibition was determined by injecting them together with 20 micrograms of aminopterin just before incubation. With this amount of the drug, only 3.5 per cent of the embryos lived longer than five days; none hatched. Folacin, vitamin B_{12}, thymine, hypoxanthine, and hypoxanthine desoxyriboside were completely without effect in changing this picture. When thymidine was injected together with aminopterin, however, toxicity of the latter was decreased to such an extent that 10 per cent of the eggs hatched, and definite shift in the direction of longer survival of those embryos which died was noted. Although ineffective by itself, injection of hypoxanthine desoxyriboside with thymidine further decreased the toxicity of aminopterin, so that 63 per cent of the embryos survived beyond five days, and 32 per cent hatched. Neither thymine nor hypoxanthine could be substituted for the corresponding desoxyribosides in these relationships. The mixture of thymidine and hypoxanthine desoxyriboside substantially duplicated the effect observed when an enzymatic digest of desoxyribonucleic acid was injected with aminopterin.

These results demonstrate the high toxicity of aminopterin for the developing chick embryo. Failure of folacin to counteract the effect of aminopterin confirms the observations of previous workers with other animals (*Nutrition Reviews* **7,** *310* (*1949*)) and with the chick embryo (D. A. Karnofsky, P. A. Patterson, and L. P. Ridgway, *Proc. Soc. Exp. Biol. Med.* **71,** *447* (*1949*); *Nutrition Reviews* **8,** *42* (*1950*)). The observation that desoxyribosides of thymine and hypoxanthine are effective in partially counteracting aminopterin inhibition indicates that in animals, as in bacteria, folacin is required for synthesis of these building stones of nucleic acid, and that these synthetic reactions are among the first to be blocked by the aminopterin-induced deficiency in folacin.

Since, by virtue of its role as a component of the genes and chromosomes, desoxyribonucleic acid is peculiarly essential to the formation of new cells, it would seem logical to expect any drug which prevented its formation to inhibit preferentially the formation of new tissue in an organism. It appears from the above that aminopterin may act, in part, in this way. Such a view may account for its striking effect, even in small amounts, in preventing initiation of embryonic development in eggs, and may also help to explain its apparently preferential inhibition of certain neoplastic growths in higher animals.

The special sensitivity of embryonic tissue to inhibition by aminopterin is pointed up anew by the work of J. B. Thiersch and F. S. Philips at the Sloan-Kettering Insti-

tute (*Proc. Soc. Exp. Biol. Med.* **74,** *204 (1950)*). These workers allowed both rats and mice to mate over a one-week period, then separated the sexes. After an additional seven days, three consecutive doses of 0.15 mg. per kilogram of body weight of aminopterin were given at twelve- to twenty-four-hour intervals, and their effects on subsequent events noted. Instead of the expected week's range in littering time, all mice born were delivered between the eighth and tenth days, and all rats between the tenth and twelfth days, following the first injection of the drug; *i.e.*, only those animals that had been pregnant eight or more days at the time of the first injection cast litters, and pregnancies of less than eight days duration were not maintained. These results were fully confirmed and extended in a series of experiments with rats in which the time of conception was controlled within forty-eight hours, and aminopterin injections made five to eight days after mating. Although implantation had occurred frequently, as proved by killing and then examining the animals, no litters were present in those animals permitted to live for twenty-three days after mating, resorption of both fetuses and placentas having taken place. Thus well-established pregnancies were interrupted by the drug. The marked sensitivity of the fetuses, as compared with the mother animal, to the drug was shown by the fact that the only symptoms of toxicity induced in the mother animals were a temporary depletion of the bone marrow and a small loss in weight.

As knowledge of the metabolic action of the vitamins increases, it may become possible to attribute the inhibitory or toxic effects of the antivitamins in animals to their interference in individual chemical reactions. Substantial progress in this direction has already been made with microorganisms, and results cited above demonstrate how, in certain favorable cases, similar results can be obtained with more complex animal systems. As a general rule, however, such interpretation is not yet possible. Whether the toxic action of small amounts of aminopterin for the mammalian embryo results from an interference with synthesis of the building stones of desoxyribonucleic acid, as it appears to in the chick embryo, cannot yet be stated, but the parallelism in the results is striking.

IMPROVEMENT OF CEREAL GRAINS

A majority of the world's population derives more than 50 per cent of the calories of its diet from cereals, chiefly wheat and rice. Most cereal grains and their products usually contain only about 8 to 10 per cent of protein, but since they are consumed in large quantity, they make a significant contribution toward the total protein intake. Since the cereal grain proteins are deficient in one or more of the essential amino acids, many attempts have been made by nutritionists to improve the protein quality of grains. This has been accomplished mainly by adding small amounts of a complete protein, such as supplied in dried milk, to the cereal products. During World War II the Germans produced large quantities of cultured yeasts as a source of good quality protein for human consumption.

B. Sure, of the Arkansas Agricultural Experiment Station, has conducted a series of studies (*Arkansas Agr. Exp. Sta. Bull.* **493** *(1950)*) on the nutritional improvement of cereal grains with small amounts of foods of high quality protein. The supplements tried were various strains of debittered brewers' and cultured yeasts, dry nonfat milk solids, low-fat soybean flour, and peanut meal.

Most of the experimental work was done with the rat, but many of the products were tested for human acceptability.

Marked increases in growth and protein

utilization were accomplished by replacing 1, 3, and 5 per cent of the enriched refined wheat flour, milled white corn meal, or polished rice, with equivalent amounts of soybean flour in the diet of rats. One or 3 per cent of cultured food yeast resulted in similar marked improvement of growth and protein utilization. When used as supplements to the proteins in milled white corn meal, the low-cost proteins from yeast or soybeans were equal to dried skim milk. Significant protein enrichment of a wheat breakfast cereal was obtained by additions of small amounts of dried food yeast, low-fat soybean flour, or dried skim milk. These protein-fortified wheat products, cooked with water, were found to be palatable in twenty-four human test meals.

Various cultured food yeasts were tried in large-scale cookery at the University of Arkansas dining halls and at the United States Veterans' Hospital at Fayetteville. One to 3 per cent of cultured yeasts was found to be acceptable in such foods as burgers, chicken pie, macaroni and cheese, sphaghetti, Spanish rice, soups, gravies, and various breads and pastries. As much as 20 per cent of such yeasts was introduced in combination with peanut butter without detection of yeast taste.

A low-cost, high-protein food was developed using dried milk solids as a base. This food contained soybean flour, vegetable shortening, calcium and iron salts, and water, and was enriched with vitamins. The processed product, which has remained stable at room temperature for over two years, contains 18 per cent of protein, 4.9 per cent of minerals, and 53 per cent of water. Its energy value is 750 calories per pound; the thiamine, riboflavin, and niacin contents, in micrograms per gram, are 12.9, 13.0, and 115.0, respectively; and it contains 3000 and 250 I. U., respectively, of vitamins A and D.

This low-cost, high-protein food was tried both as a meat extender and as a meat substitute in over three thousand test meals among college students, and also among civic organizations and representatives from various government agencies. The product was found to be more acceptable when used as a meat extender than when used as a meat substitute. It blends well in texture, flavor, and taste with ground beef, lamb, chicken, and fish in the proportion of one part meat or fish to one part of the protein food. It was estimated that the product would definitely be a low-cost protein food because its retail sale value should be less than one-half the cost of hamburger meat.

The average American diet contains about 85 g. of protein, of which about 55 per cent is animal in origin (calculated from the average per capita food consumption data of the *U.S.D.A. Misc. Pub.* **691** (*1949*)). This would appear to be a sufficiently liberal protein intake to meet requirements. On the other hand, it is well known that animal products are usually more expensive than grain products, and for this reason it is of interest to explore practical means of providing low-cost foods that furnish proteins of high nutritive quality.

RIBOFLAVIN AND OPHTHALMIA IN HORSES

The possible relation of nutrition to periodic ophthalmia (sometimes called moon-blindness) in horses was discussed several years ago by some army veterinarians (T. C. Jones, F. D. Maurer, and T. O. Roby, *Am. J. Vet. Res.* **6**, *67* (*1945*)). A detailed description of this condition in horses was published by Jones (*Ibid.* **3**, *45* (*1942*)). Catarrhal conjunctivitis in one or both eyes is usually the first indication of an acute attack of this disease. This is accompanied by photophobia and lacrimation. Repeated attacks affect the retina, lens, and ocular fluids and often cause impaired vision or blindness.

In the 1945 publication these workers emphasized the striking similarities between equine periodic ophthalmia and riboflavin deficiency in experimental animals. These similarities suggested to them that there might be a common etiology. They noted in particular that corneal vascularization was always present in periodic ophthalmia and that cataracts were also often present. The role of ascorbic acid was considered since a severe reduction of this vitamin occurred in the ocular fluids of the affected horses. It was speculated that riboflavin might be concerned in the synthesis of ocular ascorbic acid.

In a paper published in 1946 (T. C. Jones, T. O. Roby, and F. D. Maurer, *Am. J. Vet. Res.* **7,** *403 (1946)*), it was reported that riboflavin effectively prevented the appearance of new cases of periodic ophthalmia in horses. In these studies it was also reported that riboflavin did not influence the course of the disease in already established cases. These studies were conducted at the Veterinary Research Laboratory, Army Remount Depot, Front Royal, Virginia. A group of 130 officers' private mounts, which had a high incidence of periodic ophthalmia in 1943 (annual rate of 109.56 per 1000) was given a riboflavin supplement. Four sections of the depot, with a mean strength of 2552 horses and a periodic ophthalmia incidence of 50.14 per 1000 during 1943, served as controls. All were mature horses and both the experimental and control animals were kept under similar environmental conditions and received the same ration. All the animals were kept in paddocks which afforded only very limited grazing. The ration consisted of the following, in pounds: timothy hay 15; oats 9; yellow corn 2; and bran 1. Part of the time during 1943, 5 pounds of alfalfa hay were fed in place of an equal amount of timothy hay. Crystalline riboflavin was added to the ration of the experimental group on November 12, 1943. The vitamin was mixed into the grain and fed at the rate of 50 mg. per horse daily, until January 1, 1944. At this time the amount was lowered to 40 mg. per horse daily and continued at this rate during 1944. The results demonstrated a very striking influence of riboflavin. Beginning in December 1943, the appearance of new cases of periodic ophthalmia abruptly stopped in the experimental group and no new cases were observed in this group during 1944. The incidence of new cases among the control animals was 38.88 per 1000 annually in 1944, or not much different from the incidence in 1943 (50.14 per 1000 annually).

In a study reported in 1949 (T. C. Jones, *J. Am. Vet. Med. Assn.* **114,** *326 (1949)*), additional evidence was presented which indicated that riboflavin supplementation of the ration was an effective means of preventing outbreaks of periodic ophthalmia in horses. During 1945, 1946, and 1947 the riboflavin supplementation of the ration was continued at the Front Royal Depot. The original procedure was modified so that during the summer months, when good pasture was available, the riboflavin supplement was either reduced or eliminated. The occurrence of new cases of periodic ophthalmia was again completely prevented. It was again reaffirmed that recurrences in established cases could not be prevented with riboflavin.

Results obtained at a civilian institution were also reported in the 1949 paper. A particularly devastating outbreak of periodic ophthalmia occurred at St. Emma's Military Institute, Rock Castle, Virginia in 1944, where 25 out of 93 horses were affected with the disease. Since previous experience had indicated that riboflavin would not cure affected animals, all but 3 of the 25 active cases were eliminated, and the remainder of the horses were each given 40 mg. of riboflavin daily with their grain ration beginning in February 1945. The results of riboflavin supplementation among the horses in this civilian institution were equally as dramatic as those reported in the Front Royal experiments. No new cases of periodic oph-

thalmia were observed during a period of eighteen months while the horses were receiving the riboflavin supplement.

In the experiments reported in 1945, the army veterinarians also attempted to establish whether the riboflavin content of the ration could be correlated with the incidence of periodic ophthalmia at various army stations. The riboflavin content of samples of feed from Fort Robinson, Nebraska, Fort Sill and Fort Reno, Oklahoma, where the incidence of the disease was low, was compared with riboflavin content of samples from Fort Bragg, North Carolina, and Front Royal, Virginia, where the incidence of the disease was much higher. Although many of the samples from the stations with a high incidence of ophthalmia were low in riboflavin, no clearcut correlation could be demonstrated. However, it was pointed out that the samples analyzed may not have been representative of the average feed consumed over extended periods. Their experiences indicate that, in general, periodic ophthalmia in horses occurs with greater frequency at the stations where heavy rainfalls occur and where, as a result, poorly cured hay, low in riboflavin, is probably often a major ration constituent. For example, grass hay cured in the dry area of Fort Robinson, Nebraska contributed about 45 mg. of riboflavin per horse daily, contrasted with only 12.2 mg. per horse daily contributed by hay cured in the more humid atmosphere at Front Royal, Virginia.

The riboflavin content of the tissues of affected and normal horses was also reported. The concentration of this vitamin was slightly, though not significantly, lower in the tissues of affected horses than normal horses.

Attempts were made to increase the incidence of periodic ophthalmia at Front Royal by feeding rations low in riboflavin to normal horses. The low riboflavin ration greatly increased the incidence of vascularizing keratitis, but did not influence the incidence of periodic ophthalmia.

Blood analyses of affected and normal horses were also reported (T. O. Roby and T. C. Jones, *Am. J. Vet. Res.* **8,** *145 (1947)*). A moderate decrease in glucose tolerance was found in horses with periodic ophthalmia. In the affected horses the serum globulins were usually increased at the expense of albumin, the albumin-globulin ratio often being as low as 0.67:1.0 as compared with the normal ratio of 1.0 to 2.0:1.0.

These extensive investigations with horses are of particular interest in view of the frequent reports that a riboflavin deficiency may be responsible for such ocular manifestations as conjunctivitis, photophobia, and lacrimation in man (*Nutrition Reviews* **2,** *120 (1944)*; **3,** *219 (1945)*; **4,** *43 (1946)*). Though much has been written on this subject, a considerable controversy still exists as to the specificity of ocular lesions as an index of riboflavin deficiency in man. Riboflavin has often been reported to be of benefit in relieving eye symptoms in man, yet the experiences with horses indicate that this vitamin is of no value in active cases of ophthalmia, but that it is remarkably effective in the prevention of eye lesions.

Although it has been suggested that riboflavin is an essential nutrient for horses (*Nutrition Reviews* **6,** *264 (1948)*), the recent studies by the army veterinarians are suggestive that this vitamin is important in the prevention of eye lesions in horses.

It is unfortunate that these extensive studies were not more adequately controlled. No control data are given in the Front Royal study during 1945 to 1947. It is therefore not known what the incidence of periodic ophthalmia would have been in the absence of riboflavin therapy. However, the very abrupt disappearance of new cases in the riboflavin-treated group in 1944, contrasted with a continued relatively high incidence among controls, suggests a very positive riboflavin effect.

NUTRITION, ORAL HYGIENE, AND TOOTH DECAY

The possible relationship of diet during tooth development to the formation of caries-resistant and caries-susceptible teeth has been studied in a number of ways (*Nutrition Reviews* **4,** *41* (*1946*); **5,** *68* (*1947*); **6,** *196* (*1948*)); likewise, investigations on the effect of various diets upon teeth which are already formed have been numerous (*Ibid.* **1,** *205* (*1943*); **3,** *160* (*1945*); **4,** *115* (*1946*)). Until recently, the potential method of caries control in human beings through simple oral hygiene procedures has never been adequately explored.

L. S. Fosdick (*J. Am. Dent. Assn.* **40,** *133* (*1950*)) has conducted an experiment in which the dental conditions of a relatively large group of college students were evaluated carefully by clinical exploration and by radiographic examination. This student body was divided into two groups. One of these groups was instructed to continue their usual habits in so far as oral hygiene was concerned. The individuals in the other group were supplied with toothbrushes and a neutral tooth paste which contained dicalcium phosphate as the abrasive, glycerol, gum, a flavoring material, a detergent, and water. The latter individuals were instructed to brush their teeth thoroughly within ten minutes after each ingestion of food or sweets and, when brushing was impossible, to rinse their mouth thoroughly. At the end of the first and second years, the individuals in the two groups were again subjected to detailed dental examinations. At the end of two years, the 276 individuals in the control group had developed an average of 2.53 clinically detectable new carious surfaces and an average of an additional 1.34 new carious surfaces observed through radiographic examination. In contrast, during the two years, the 429 subjects in the experimental group had developed an average of 1.49 new carious surfaces which were clinically detectable, and an average of 0.53 new carious surfaces which were detectable by radiographic examination. These figures represent a decrease of 41 and 60 per cent, respectively, for the two categories of tooth decay.

In another experiment concerning oral hygiene, the effect of using a tooth paste containing penicillin was compared to the use of the same tooth paste without penicillin (H. A. Zander, *J. Am. Dent. Assn.* **40,** *569* (*1950*)). The study was conducted in grades 1 to 6 in two elementary schools. In the school in which the plain tooth paste was used, 174 children participated; in the other school, 235 children used tooth paste into which 500 units of potassium penicillin had been incorporated per gram. At the beginning of the experiment, all children were examined clinically and with the aid of radiographs. During the school session, the children of both schools brushed their teeth once daily in their classrooms under the supervision of the teacher or dental hygienist. They were instructed to use the tooth powders at home, night and morning. At the end of one year, 162 children in the control school and 216 in the penicillin school were re-examined and, at the end of the second year, 150 children in the control school and 202 in the penicillin school were available for re-examination. The incidence of caries on previously caries-free surfaces during the first year was 55.3 per cent less in the group using the dentifrice containing penicillin and during the second year was 57.6 per cent lower in the same group. Since there was no control group whose individuals did not brush their teeth regularly in this experiment, there is no record of whether the brushing of teeth with the plain dentifrice was of any value. The only adverse effects observed in the two-year study were dryness and cracking of the lips in the corner of the mouth. These symptoms were observed soon after introduction of the powder in 6 children from the penicillin school and 13 from the control school. The lesions dis-

appeared within two or three days after use of the powder was discontinued and only reappeared in 2 children from the penicillin school who had a recurrence of the lesions each time the tooth powder was used. No more penicillin-resistant bacteria were observed in the saliva of the children who had used the penicillin tooth paste than in the saliva of children using the plain dentifrice. From the standpoint of the etiology of tooth decay, the most interesting point demonstrated in this study was contained in a later publication (R. J. Fitzgerald, H. A. Zander, and H. V. Jordan, *J. Am. Dent. Assn.* **41,** *62* (*1950*)). Although the incidence of tooth decay had apparently been greatly decreased by the use of penicillin tooth paste throughout the experimental period, the salivary lactobacillus counts were not significantly different from the average value for the control group of children. According to the opinion of many dental investigators, salivary lactobacillus counts are an accurate index of dental caries activity and, indeed, lactobacilli have been suggested by some researchers as one of the major offenders in the production of tooth decay. Thus, it would seem that a fundamental point had been demonstrated in this experiment, namely, that lactobacillus counts may remain high even when dental caries activity has been greatly depressed.

Whether an antibiotic as important otherwise as penicillin can be safely used repeatedly and routinely for the control of tooth decay remains to be demonstrated more conclusively. At least, these two groups of experiments demonstrated that, in addition to the benefit to the teeth of satisfactory procedure during tooth development and after tooth eruption, maintenance of good oral hygiene can materially affect the health of the teeth but, in so doing, does not, necessarily, alter the bacterial flora of the oral cavity.

INSECT NUTRITION AND THE VITAMIN T-COMPLEX

An article by J. Leclercq (*Arch. internat. de physiol.* **57,** *350* (*1950*)) that confirms and extends previous results again calls attention to the interesting properties of vitamin T, a water-soluble growth factor in the nutrition of insects.

Insect nutrition has been the object of but scant work as compared to mammalian or bacterial nutrition. Some aspects of this branch of nutrition have been previously reviewed (*Nutrition Reviews* **3,** *173* (*1945*); **7,** *7, 219* (*1949*)). It may nevertheless be useful to recall that McCay and his collaborators had been able to show that at least certain insects did not require or synthesize vitamin A (M. F. Crowell and C. M. McCay, *Physiol. Zool.* **10,** *368* (*1937*); R. E. Bowers and McCay, *Science* **92,** *291* (*1940*)). Similarly, M. Lafon and G. Teissier (*Compt. rend. Soc. de biol.* **131,** *75* (*1939*)) and H. E. Martin and L. Hare (*Biol. Bull.* **83,** *428* (*1942*)) showed that larvae of *Tenebrio Molitor* did not require vitamins A, C, D, E, K, or choline, but required cholesterol, as well as thiamine, riboflavin, pyridoxine, niacin, and pantothenic acid. Other substances contained in yeast or liver extracts seemed to stimulate growth.

G. Fraenkel and M. Blewett (*Biochem. J.* **41,** *xviii* (*1947*); *Nature* **157,** *697* (*1946*)) showed that one of these factors was folacin; another one, also water soluble, was not identical with any of the known vitamins and was designated B_t by them.

Independently, W. Goetsch (*Oesterr. Zool. Z.* **1,** *193* (*1947*)), described a new vitamin which he called vitamin T, a growth factor in insect nutrition. Vitamin T is found in various kinds of yeast, of the genus *Torula* in particular, and in ascomycetes like *Penicillium* and *Hypomycetes*, as well as in insects which feed on these microorganisms, ter-

mites in particular. It is soluble in water and ethyl alcohol and resistant to heat.

Goetsch (*Experientia* **3,** *326 (1947)*) showed that while vitamin T was not indispensable for maintenance of or even for some growth in insects, it increased considerably both their growth rates and their efficiency in food utilization and stimulated oxygen consumption. When large amounts of the vitamin were administered, abnormally large insects were produced in the species *Periplaneta orientalis*, *Tachycines asynomorus*, and *Drosophila melanogaster*. Furthermore, the resultant gigantism was accompanied by disproportions in growth. The head and the mandibles were stimulated to grow proportionately much larger than the body as a whole. In *Drosophila* fed large amounts of vitamin T the eyes in particular became extraordinarily large as compared with controls not fed the vitamin. In ants, administration of vitamin T not only altered the body proportions but also their habits. Those receiving vitamin T worked outside the nest while controls of the same age tended to work in the nest chamber.

Concomitant with changes in size and habits, it was observed that the pigments of insects receiving vitamin T were more intense than those of their controls. It must be noted that appearance of these changes, particularly those associated with gigantism, requires two sets of conditions: (1) a liberal supply of protein in the diet; (2) that vitamin T be fed during the early stages of development.

J. Leclercq (*Biochim. et biophys. acta* **2,** *329 (1948)*) was able to analyze this point even more accurately by determining the particular stages of development when vitamin T was required. During some stages, vitamin T is not required. In his latest published study (*Arch. internat. de physiol., loc. cit.*) he was able to multiply the weight of the larvae by a factor of three after thirty-four days. It must be noted that Goetsch had pointed out (*loc. cit., 1947*) that if excessive amounts of vitamin T are administered, the effect on maturation becomes more important than that on size, with consequent speeding up of development and resulting production of dwarves rather than giants.

That vitamin T may be a substance of significant importance in the metabolism of mammals is indicated by the clinical reports of Goetsch (*loc. cit., 1947*) on the favorable effects of administration of vitamin T in certain skin conditions, as well as by the studies of E. Kupka (*Z. f. Vitaminforsch.-Hormone.- u. Fermentforsch.* **2,** *187 (1949)*) on the increased resistance induced by vitamin T to the toxicity of small doses of strychnine in rats. Finally, G. Nugbaumer (*Med. Klin.* **44,** *636 (1949)*) reported favorable effects, after administration of vitamin T, in the treatment of certain growth disturbances in children. Gains in weight, in red cell counts, and in hemoglobin contents were observed.

It seems unnecessary to elaborate on the various points which have to be elucidated before vitamin T can be accepted as a known vitamin of the B group, distinct from any other member of the B-complex and with well-established and measurable chemical and physiologic properties. However, it can be pointed out that the case of vitamin T is but an example of the special problems of invertebrate nutrition, where, to the familiar aspects of growth, reproduction, and maintenance are added those of drastic alterations of forms and modes of life. With these metamorphoses are probably associated changes in nutritional requirements. The unraveling of these variations would shed light, not only on problems of insect biology, but also on many general and fundamental aspects of biology.

PRELIMINARY REPORTS FROM NEWBURGH-KINGSTON

With the appearance of two preliminary reports (D. B. Ast, S. B. Finn, and I. McCaffrey, *Am. J. Pub. Health* **40,** *716 (1950)*; E. R. Schlesinger, D. E. Overton, and H. C. Chase, *Ibid.* **40,** *725 (1950)*) of the observations during the first three years of the Newburgh-Kingston study, the tantalizing data make one impatient for the lapse of additional time to allow final appraisal of the effectiveness of the addition of fluorides to domestic water supply for the reduction of dental caries.

This classic public health study was planned as a result of a series of remarkable epidemiologic investigations which began in 1908, with the investigation of the cause of mottled enamel, by the Colorado Springs Dental Society. The now well-known relationship between mottled enamel and the presence of excessive quantities of fluorides in the drinking water was proved through a great number of epidemiologic observations followed by studies of the effects of changed water supply and corroborated by laboratory investigations on animals (*see* "Fluorine and Dental Health," edited by F. R. Moulton, *Am. Assn. Advancement Sci., Washington, D. C. (1942)*).

Incidental to these studies it was noted that dental caries experience was highest in communities whose water supply was free of fluorides and that the caries experience approached a minimum in communities whose water supplies contained approximately 1.0 to 1.5 parts per million of fluorine. No significant mottling occurs with these lower concentrations of fluoride (*see* "Dental Caries and Fluorine," edited by F. R. Moulton, *Am. Assn. Advancement Sci., Washington, D. C. (1946)*).

Despite the strongly suggestive evidence that fluorine was the factor responsible for this reduction in dental caries, it remains an hypothesis until proved by observing the crucial experiment in which there are controlled observations of the effect of an increase in the fluorides in a variety of water supplies upon the prevalence of dental caries. The intensive Newburgh-Kingston experiment and the more limited and less well-controlled studies in several other communities will serve this purpose.

The design of the Newburgh-Kingston study is worthy of comment. Two cities were selected, each of approximately 30,000 population and situated on the Hudson River but separated by some thirty miles and with similar climate and fluoride-free water supplies. The Kingston community agreed to serve as a control, while sodium fluoride was added to the water supply of Newburgh. Basic dental examinations were started in June 1944, and it was determined that the caries rates were comparable between similar groups in the two communities. Beginning on May 2, 1945, sodium fluoride was added to the water supply of Newburgh in quantity sufficient to maintain a fluoride content of 1.0 to 1.2 p.p.m. Successive dental examinations are made at yearly intervals in the two cities. The examinations in Newburgh have all been carried out by one examiner. The basic examination in Kingston was done by the Newburgh examiner, while later surveys in Kingston have been conducted by two dental hygienists trained by the Newburgh examiner.

Inasmuch as ingestion of large quantities of fluorides is known to result in toxic manifestations (mottled enamel, bone changes, etc.), every effort has been made to ascertain the safety of the procedure of fluoridation of the water supply. Despite extensive epidemiologic evidence of the complete safety of the amounts of fluoride added, this aspect of the problem has been examined. Careful periodic health evaluations were made of a group of children in each city prior to the date of introduction of fluoride into the water supply at Newburgh. Five hundred children, similarly distributed in age groups from 1 to 9 years, from each com-

mites in particular. It is soluble in water and ethyl alcohol and resistant to heat.

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munity were examined. This examination included a careful medical history and an annual physical examination with especial emphasis upon those organs which might conceivably be affected by fluoride. In Newburgh a number of routine laboratory determinations are made at each visit. In addition, roentgen films are taken to determine whether there may develop changes in bone density (osteosclerosis is a finding of chronic fluoride intoxication). Careful examinations are also made of the eyes and ears of a special study group of 25 children. Finally, special studies of fluoride excretion in diseased states are under way. The thoroughness of this effort is impressive. Indeed, the intensiveness of the systemic survey is far greater than that of any previous survey in communities where the water supplies in their natural state contained fluorides supplying 1.0 to 1.2 p.p.m. of fluorine.

In agreement with epidemiologic findings, these careful checks on possible toxicity have revealed that there has been no significant deviation in any of the factors studied between the initial base-line examinations prior to the introduction of fluoride into the drinking water and three years afterward. Thus, the study indicates that ingestion of added fluoride in the quantities of 1.0 to 1.2 p.p.m. in the water supply has proved a safe procedure for this three-year period. The workers conservatively caution that "Final conclusions regarding the possible systemic effects of fluoride in the dosage employed should not be drawn before termination of the 10-year study."

The present analysis of the effect on caries rate is limited to: (1) children aged 6 to 12 years who were in the original base study and who had received successive examinations to the age of 12 years, and (2) children who have reached the age of 6 years since the beginning of the experiment and who have been available for successive examination. This embraces approximately 3400 children from Newburgh (fluoride) and 2800 from Kingston (control).

The proportion of erupted permanent teeth with evidence of caries experience (*i.e.*, decayed, missing, or filled teeth; abbreviated as DMF) decreased in each successive examination period in Newburgh from 21 per 100 prior to fluoridation to 14.8 per 100 at the three-year mark. By contrast, in Kingston the DMF rate remains at approximately 21 for all three years. This suggests that a 30 per cent improvement has occurred in the DMF rate in Newburgh.

The observed difference in caries prevalence between the two cities varied considerably according to the type of tooth. A lowering by 18 per cent of the DMF was observed in first molars while a decrease of 55 per cent was noted for the central and lateral incisors. Thus, the greatest saving observed to date resulted in those teeth least frequently attacked by caries. In this regard, the experimental results are almost identical with those obtained in the widespread epidemiologic surveys.

The fact that the teeth of the younger children had benefited more by fluoridation of the water supply than the teeth of the older children is noteworthy. For example, in 1949, the 6½ and 7½-year-old Newburgh children who were 3 and 4 years old, respectively, at the beginning of the experiment had about 50 per cent less tooth decay than the 6½ and the 7½-year-old Kingston children. In contrast, the 12½-year-old Newburgh children who were 9 at the beginning of fluoridation had only 3.9 per cent less tooth decay than the 12½-year-old Kingston children. Newburgh children in the intermediate age categories between 7½ and 12½ years had benefited more than the 12½-year-olds but much less than the 6½ and 7½-year-olds. These differences in the response of teeth of children of varying ages were not unexpected. Survey data have strongly suggested that the major beneficial effect occurred when the fluorides were ingested

during the calcification and maturation of the teeth and that erupted teeth benefited only slightly from exposure to waters bearing fluorides.

Ast, Finn, and McCaffrey (*loc. cit.*) caution that it will be necessary to continue collecting data through the total ten-year period (through 1956) to obtain additional information concerning the prophylactic value of fluoridation of water supply.

These admittedly preliminary but encouraging reports emphasize the potential importance of this study. The farsighted communities of Newburgh and Kingston are to be congratulated for their willingness to undertake this objective appraisal of what promises to be a most important public health procedure. Whatever the results of this study, all cities will owe these two communities a great debt indeed.

In several other widely separated parts of this continent where presumably the composition of the water supplies varies considerably, dental surveys are being conducted after artificial fluoridation of the drinking water for periods of approximately three years. The Fluorine Study Committee of the Wisconsin State Dental Society has summarized the results obtained in Brantford, Ontario, Sheboygan, Wisconsin, and Marshall, Texas (*J. Wisconsin State Dent. Soc.* **26,** *6 (1950)*). After forty months of fluoridation at a level of 1.3 p.p.m. of fluorine, the 5-year-old children in Brantford, Ontario had 47 per cent less tooth decay than children of the same age at the beginning of the experiment. All children between the ages of 5 and 16 showed an average improvement of 22 per cent as compared with the average caries experience at the beginning of the trial. In Sheboygan, Wisconsin, after forty-four months of fluoridation at a level of 1 p.p.m., the kindergarten children and the fourth and eighth grade students had averages of 39.6, 24.4, and 18.0 per cent, respectively, lower dental caries experiences than children in the same grades at the beginning of the study. After twenty-nine months of fluoridation at an unstated level, the children of "all" ages in Marshall, Texas, had 23 per cent less tooth decay than children in the same age group prior to the addition of fluorides. Among the 6-year-old children, there was a reduction of 47 per cent. The similarity of the results in these three surveys to those obtained in the Newburgh-Kingston experiment greatly strengthen the case for the artificial addition of fluorides to water supplies as a means of reducing the incidence of tooth decay. It is to be noted in connection with these three cities that unlike the Newburgh-Kingston experiment, no reports have been made of any concurrent studies of the possible harmful systemic effects of fluorides. At this early stage in the knowledge of safe levels for fluoride ingestion, any survey conducted without studies of possible systemic sequelae is open to criticism. Even in the intensive experiment at Newburgh, no statement was made of any radiographic studies of the vertebral column, which according to K. Roholm, the eminent Danish toxicologist, was the most sensitive area of the skeletal system in so far as fluorosis was concerned ("Fluorine Intoxication," *H. K. Lewis and Co., Ltd., London (1937)*).

As an example of the growing public interest in fluoridation of public water supplies, F. A. Bull (*J. Am. Dent. Assn.* **41,** *146 (1950)*) cites evidence that 80 per cent of the urban population in the state of Wisconsin live in communities which have begun to add fluorides to the drinking water, or are in the process of installing the necessary equipment. In all, at the time this article was published, twenty-seven Wisconsin cities, including the state capitol, were adding fluorides and an additional thirty-seven, including Milwaukee, had approved fluoridation and were in the process of equipment purchase and installation.

Another public health approach to re-

duction in dental caries by use of fluorides is the topical (local) application of 2 per cent sodium fluoride to the teeth of children. This procedure must be carried out by trained personnel, either a dentist or a properly trained dental hygienist. The procedure, when properly carried out, and when repeated at approximately three-year-intervals until maturity is attained, is reported to result in a caries rate reduction of some 40 per cent (L. S. Kleinschmidt, *Am. J. Pub. Health* **40,** *713 (1950)*).

The eightieth Congress of the United States appropriated funds for conducting demonstrations in each of the states and several territories of the approved technic for the topical application of fluoride. In August 1948, personnel for several teams was assembled by the U. S. Public Health Service and the program of treatment was begun in collaboration with local health groups, dentists, and others in several communities. The organization of these "Fluoride Demonstration Units" and the role of the community are described in two reports of interest to public health workers (W. P. Kroschel, *Am. J. Pub. Health* **40,** *708 (1950)*; Kleinschmidt, *loc. cit.*).

These teams have two functions: (1) demonstration to dentists, dental hygienists, and the local health personnel of the approved technic for application of sodium fluoride, and (2) stimulation of local fluoride programs.

Despite the effectiveness of the method of topical application of fluoride, reflection upon the personnel required for mass treatment (an average operation in a community project may be expected to treat 2500 to 2800 children annually) indicates the practical limitations of the approach. The total cost of the fluoridation of water supplies has varied from 9 to 11 cents per capita annually (Bull, *loc. cit.*). If fluoridation of water supplies proves to be equally effective, it may provide a much less costly public health approach for communities with public water supplies.

THE CITROVORUM FACTOR

Following the discovery of vitamin B_{12} in liver, there developed without delay various methods of quantitative estimation of this factor. One of the useful assay procedures is a microbiologic method employing *Lactobacillus lactis* Dorner; soon it was observed that *Lactobacillus leichmannii* also requires vitamin B_{12} for growth and this organism has been suggested for the microbiologic assay (H. R. Skeggs, J. W. Huff, L. D. Wright, and D. K. Bosshardt, *J. Biol. Chem.* **176,** *1459 (1948)*).

Further investigations into the nutritional requirements of the lactic acid bacteria have disclosed additional complexities in this regard. Thymine desoxyriboside (thymidine), which can replace vitamin B_{12} in the nutrition of *L. lactis* Dorner, is also required by *L. leichmannii* and *Leuconostoc citrovorum* (E. E. Snell, E. Kitay, and W. S. McNutt, *J. Biol. Chem.* **175,** *473 (1948)*).

On further examining the needs of *Leuconostoc citrovorum*, H. E. Sauberlich and C. A. Baumann (*J. Biol. Chem.* **176,** *165 (1948)*; *Nutrition Reviews* **8,** *282 (1950)*) came to the conclusion that, whereas this organism grew well on certain liver extracts, there were unpredictable variations between the antipernicious anemia potency and the growth stimulation for *Leuconostoc citrovorum* in these extracts. Both folacin and thymidine stimulated growth, but not so well as liver extract.

Another indication that liver extract contains multiple stimulators of the growth of lactic acid bacteria is the observation that when 4-amino-pteroylglutamic acid or methylfolic acid is added to the medium,

growth of *Leuconostoc citrovorum* is retarded; concentrates of citrovorum factor from rat urine or from liver extract reverse this toxicity very effectively, whereas either folacin or thymidine is much less effective and vitamin B_{12} was completely ineffective (H. E. Sauberlich, *Arch. Biochem.* **24,** *224 (1949)*; *Nutrition Reviews* **8,** *282 (1950)*; **9,** *12 (1951)*).

From these various observations there has grown the conviction that the so-called citrovorum factor and vitamin B_{12} are not identical, particularly since pure vitamin B_{12} will not stimulate the growth of *Leuconostoc citrovorum* (H. E. Scheid and B. S. Schweigert, *J. Biol. Chem.* **185,** *1 (1950)*).

Various technical devices have recently been employed to separate the citrovorum factor from the antipernicious anemia factor in liver. Thus, one group (H. P. Broquist and co-workers, *Proc. Soc. Exp. Biol. Med.* **71,** *549 (1949)*) was able to separate two fractions from liver extract by the use of filter paper chromatography; one of these fractions, presumably thymidine, supported growth of both *L. leichmannii* and *Leuconostoc citrovorum*, whereas the other fraction stimulated only *Leuconostoc citrovorum*. As had been observed previously, folacin and thymidine both elicited a modest growth response in *Leuconostoc citrovorum* when used separately; on the other hand, used together in the medium they produced a marked response.

Liver extract (reticulogen) has been electrolyzed in 0.02 M acetic acid (C. M. Lyman and J. M. Prescott, *J. Biol. Chem.* **178,** *523 (1949)*); under these conditions the citrovorum factor migrated to the positive electrode and was thus separated from the factor or factors stimulating *L. leichmannii*. Liver extract was also treated with anion exchange resin IRA 400 and the citrovorum factor was quantitatively retained on the column whereas all other active fractions passed through (Lyman and Prescott, *Fed. Proc.* **8,** *220 (1949)*). Although the eluate and the adsorbate differed markedly in their resistance to autoclaving in an alkaline medium, it was concluded that they seemed to be related chemically.

Doubtless, some of the apparent contradiction regarding the growth-promoting potency of various fractions of liver and of other preparations for specific lactic acid bacteria arises from their lack of chemical homogeneity. Thus, it has been concluded that the presence of purines and pyrimidines in the fractions employed alter the efficacy of antipernicious anemia fractions and of folacin in supporting growth of *Leuconostoc citrovorum* (H. E. Sauberlich, *Fed. Proc.* **8,** *247 (1949)*; *Arch. Biochem.* **24,** *263 (1949)*). Large amounts of folacin were effective in this study if purines were also present. Even the action of the citrovorum factor itself appears to be conditioned by guanine, hypoxanthine, or xanthine, the latter being the most potent of the purines. The free purine is more active than the nucleoside or nucleotide and it cannot be replaced as a supplement to the citrovorum factor by vitamin B_{12}, folacin, thymidine, or folacin plus thymidine.

As further work on the citrovorum factor is carried out, it is apparent that a relationship exists between this growth stimulant and folacin (*Nutrition Reviews* **8,** *282 (1950)*). Of particular interest in this respect is the observation (H. E. Sauberlich, *J. Biol. Chem.* **181,** *467 (1949)*) that when folacin is administered to rats and to human subjects, citrovorum factor appears in the urine in proportion to the amounts of folacin administered. Possible mediation of this phenomenon by the intestinal organisms was ruled out and it was shown that the effective citrovorum factor in the urine is not identical with folacin.

Biochemical studies on the distribution of the citrovorum factor have served to characterize it further (Scheid and Schweigert, *loc. cit.*). Using crystalline vitamin B_{12} as a

standard, injectable liver extract, beef liver, and beef round were compared with respect to the presence of growth stimulants for *L. leichmannii* 313 and 327 and *Leuconostoc citrovorum* 8081. The potencies of these three substances are similar when measured (titration of produced acid) with *L. leichmannii* as the test organism, but this vitamin B_{12} potency and the growth-stimulating factor for *Leuconostoc citrovorum* were not parallel. The citrovorum factor is more resistant to autoclaving in acid or alkaline medium than is vitamin B_{12} and there is no evidence of the freeing of bound form of citrovorum factor through such treatment. On the other hand, digestion with pancreatin and erepsin did release the citrovorum factor from beef liver.

In another study (H. P. Broquist, E. L. R. Stokstad, and T. H. Jukes, *J. Biol. Chem.* **185,** *399* (*1950*)) a purified citrovorum factor concentrate was made from a liver fraction derived from the alcohol-insoluble residue of a water extract of liver, through adsorption on charcoal at pH 4.0 and elution with ethanol-ammonia-water mixture. This concentrate of citrovorum factor reversed the inhibitory effect of 4-amino-pteroylglutamic acid on *Leuconostoc citrovorum*, on *Streptococcus faecalis*, and on the growth of mice. The requirement for citrovorum factor in this regard is reduced by thymidine. The distribution of citrovorum factor activity and of folacin activity from a liver fraction between n-butanol and water at pH 2.0 is identical within the limits of the microbiologic assays. Competitive antagonism studies, microbiologic growth stimulation, and physicochemical investigation suggest that the citrovorum factor and pteroylglutamic acid are chemically related (*Nutrition Reviews* **9,** *12* (*1951*)).

SELF SELECTION OF DIET BY RATS

In recent years studies at the University of Pittsburgh by E. M. Scott and his associates have demonstrated that rats possess only a limited ability to develop an appetite for essential nutrients in which they are deficient. These results strongly indicate that appetite therefore cannot be relied upon as a means of obtaining good nutrition.

The eating behavior of animals, given a choice of diets varying in nutritional adequacy, has been reviewed several times (*Nutrition Reviews* **2,** *199* (*1944*); **5,** *24* (*1947*); **6,** *29* (*1948*); **7,** *175* (*1949*)).

Two recent reports from the Pittsburgh laboratory are concerned with the appetite for minerals of normal and mineral-deficient rats. These two papers are the tenth and eleventh in a series dealing with self selection of diet. The tenth paper in the series (E. M. Scott, E. L. Verney, and P. D. Morissey, *J. Nutrition* **41,** *173* (*1950*)) deals with the appetite of rats for sodium, chloride, and sodium chloride; and the eleventh, by the same authors (*Ibid.* **41,** *187* (*1950*)), is concerned with appetites for calcium, magnesium, and potassium.

The basal diet used consisted of 10 per cent hydrogenated vegetable oil, 24 per cent vitamin-free casein, 62 per cent sucrose, and 4 per cent salt mixture. Vitamins were administered daily in the form of pills. The mineral-deficient diets differed from the above diet only in the salt mixture which was modified to render it deficient in the element under study. For example, in the sodium-deficient diet the sodium chloride was replaced by potassium chloride.

The experiments were conducted as follows: During a pre-experimental period of three weeks, 10 weanling rats were given the basal diet in each of two feed cups, and an additional 10 weanling rats were given the diet deficient in a given element in each of two feed cups. During this period the animals were weighed daily and the amount of food eaten from each cup was determined.

The cups were interchanged daily in a predetermined random manner. Following the pre-experimental period both control and deficient animals were again offered two cups of food, but now one contained the deficient diet and the other contained the adequate diet. The cups were again interchanged daily in the same predetermined manner and the amount of food consumed from each was determined. This procedure thus made it possible to compare the eating behavior of both control and deficient animals when offered a choice of the normal diet and the deficient diet.

If the animals exhibited an appetite for a given element in which they were deficient, these investigators then attempted to establish whether the appetite was learned or whether the appetite for the missing factor represented a true hunger. They used a previously developed technic (E. M. Scott and E. L. Verney, *J. Nutrition* **34,** *471 (1947)*) by which they showed that appetites for certain vitamins are acquired by learning and do not represent a true hunger. Briefly, the technic consisted of allowing rats, both normal and deficient, an opportunity to associate a flavor, or lack of it, with the food essential under study. The flavor used was anise, a flavor which these workers had shown to be neither preferred nor avoided.

The general plan was to allow animals their choice of two diets, one (A) flavored and the other (0) not flavored. The flavored diet contained the element under study, the unflavored diet did not. After a few days, the choices were changed so that the flavored diet (A) did not contain the element under study but the unflavored diet (0) did contain the element. If the animals continued to eat the flavored diet, then it was considered that the appetite for the element under study was learned; in this case the learning was through association with a flavor. On the other hand, if the animals did not continue to eat the flavored diet, but switched to the unflavored diet, then it was considered that the appetite for the element under study was a true hunger and thus could not be influenced by the flavor.

The results are summarized as follows: Both normal and sodium-deficient rats preferred the diet containing between 0.02 and 0.05 per cent sodium chloride over the diet containing approximately 0.002 per cent sodium (the sodium-low diet). When the diet with sodium contained between 0.1 and 2.5 per cent sodium chloride, neither sodium-deficient nor normal rats showed any preference for this diet over the sodium-low diet. At high concentrations of dietary sodium chloride (5 per cent or 12 per cent), both normal and sodium-deficient rats preferred the sodium-low diet. Sodium deficiency thus could not be demonstrated to lead to a change in salt appetite. This result challenges the belief that animals seek out the "salt-lick," or that rodents chew clothing tainted with perspiration in preference to unsoiled clothing because they are hungry for salt.

The results of tests to establish whether rats develop an appetite for chloride were inconclusive.

One result for which Scott and his associates had no explanation was the obvious appetite of sodium-deficient animals for sodium bicarbonate, but not for sodium dihydrogen phosphate or sodium sulfate.

The results, on the whole, emphasize that it is difficult to demonstrate an appetite for sodium by sodium-deficient rats. In contrast with this, it has been reported by several investigators that the increase in salt need in adrenalectomized rats leads to an increase in salt appetite (C. P. Richter, *Am. J. Physiol.* **115,** *155 (1936)*; J. Mark, *Endocrinology* **31,** *582 (1942)*; W. G. Clark and D. F. Clausen, *Am. J. Physiol.* **139,** *70 (1943)*).

Calcium-deficient rats were found to develop a preference for diets with calcium over calcium-low diets. This was true, whether the calcium salt was the carbonate, the dibasic phosphate, or the sulfate. The result with calcium carbonate was unusual in one respect in that both control and

deficient animals preferred diets containing comparatively large amounts of this substance.

Magnesium-deficient rats tended to avoid diets containing magnesium to a greater extent than did control animals, and the magnesium avoidance involved a learning process. Three magnesium salts were included in the test, namely, the carbonate, the sulfate, and the oxide.

The results with potassium indicated that animals deficient in this element do not develop an appetite for diets containing potassium dihydrogen phosphate. Magnesium and potassium deficiencies developed promptly. It was therefore necessary, in the experiments with these elements, to limit the pre-experimental period to two weeks instead of the usual three weeks.

The results of the flavor studies in the mineral deficiencies were not as uniformly consistent as they were in the earlier reported vitamin-deficiency studies. For example, in the calcium experiments, the rats learned to associate the effects of calcium with flavor, or lack of it. On the other hand, the results with the sodium experiments were inconclusive. Two of the sodium salts, bicarbonate and chloride, gave results which could be interpreted as indicating that the animals were influenced more by the flavor of the salt than by the flavor of anise.

From the results reported by Scott and his associates, it is obvious that rats do not possess the ability to self-select diets compatible with good mineral nutrition. These workers have summarized the results of all the controlled experiments reported in the literature dealing with appetite in relation to need for specific dietary essentials. Of the twelve different dietary essentials studied, an appetite for five has been demonstrated, for two the results were doubtful, and for five no appetites could be shown. Those for which rats develop an appetite as a result of need are thiamine, pyridoxine, riboflavin, calcium, and sodium (sodium bicarbonate). Those for which the appetites have been demonstrated, but for which the results are doubtful, are pantothenic acid and chloride. Those for which rats do not develop an appetite, in the presence of a need, are vitamins A and D, protein, potassium, and magnesium.

These workers believe that for those specific nutritional factors for which appetites can be demonstrated, the appetite is a learned one rather than a true hunger.

In their studies with the vitamins (E. M. Scott and E. Quint, *J. Nutrition* **32,** *285 (1946)*; Scott and Verney, *loc. cit.*) it was noted that appetites are not developed immediately but require several days, and further that appetites are not developed by all deficient animals. They consider this as evidence that appetites are learned. The experiments with flavors also substantiate the view that learning is involved.

These interesting results with rats suggest that man's eating habits and those of the rat are broadly similar in that neither apparently possesses any involuntary drive to eat diets that supply missing dietary factors. Man's nutrition is radically influenced by acquired habits, with no obvious relation to nutritional need, as is the case, for example, when large population groups prefer polished rice to the more nutritionally adequate unpolished rice.

FACTORS THAT INFLUENCE THE NUTRITIVE VALUE OF LEGUME SEEDS

The nutritive value of a number of legume proteins can be improved by heat treatment. In previous discussions (*Nutrition Reviews* **3,** *238 (1945)*; **4,** *104 (1946)*; **5,** *338, 351 (1947)*; **8,** *193, 218 (1950)*) it was indicated that the heat treatment of soybeans in-

creased the nutritive value of the proteins by destroying an enzyme inhibitor (trypsin inhibitor) and that this was paralleled by an increased availability of the sulfur-containing amino acids, cystine and methionine.

Although the heat destruction of trypsin inhibitor appears to be correlated with improved nutritive value of soybean protein, this relationship does not appear to be true for all types of legumes. This is evident from the data published by R. Borchers and C. W. Ackerson of the Nebraska Agricultural Experiment Station (*J. Nutrition* **41,** *339* (*1950*)). These workers compared the nutritive value of raw and autoclaved legume seeds of eleven different species and found no consistent relationship between the effect of autoclaving on nutritive values and the presence or absence of trypsin inhibitor in the raw seed.

The Nebraska workers used as a standard heat treatment, autoclaving at 15 pounds pressure for thirty minutes. The raw and heat-treated products were fed to litter-mate weanling rats for a twenty-day test period in a diet of the following percentage composition: Protein (raw or treated legume seed, N × 6.25), 12; DL-methionine, 0.6; salt mixture, 2; choline chloride, 0.5; cod liver oil, 5; lard, 10; and corn starch to make 100. Further vitamin additions (milligrams per 100 g. of diet) included thiamine hydrochloride, 0.5; calcium pantothenate, 1.25; niacin, 2.5; pyridoxine, 0.125; and riboflavin, 0.5.

The legume seeds studied were about equally divided as to those which were and those which were not improved in nutritive value by autoclaving. Furthermore, there was clearly no correlation between improvement in nutritive value after autoclaving and the presence or absence of trypsin inhibitor. The nutritive value of the protein of velvet, lima, common, and soy beans was improved by autoclaving, and these legumes contained the trypsin inhibitor. Jack bean, lentil, and horse bean, on the other hand, were improved by autoclaving, but did not contain the inhibitor. The nutritive value of the peanut, partridge pea, chick pea, sweet pea, lespedeza, and mung bean were not improved by heating, but contained the trypsin inhibitor, and guar, common pea, or common vetch were not improved by autoclaving and did not contain the trypsin inhibitor. The concentration of trypsin inhibitor in various legume seeds was previously reported by the Nebraska workers (R. Borchers and C. W. Ackerson, *Arch. Biochem.* **13,** *291* (*1947*)).

In general, the results of the feeding tests agree well with those reported in the literature. However, the report of L. R. Richardson (*J. Nutrition* **36,** *451* (*1948*)) that the black-eyed pea is not improved by heating was contradicted by the results of the Nebraska workers. In a more extensive study they observed marked improvement of nutritive value of black-eyed peas with fifteen, thirty, or sixty minutes of autoclaving. Autoclaved black-eyed peas were also superior to the raw peas when the methionine was omitted from the diet.

The results of the Nebraska workers substantiate the view that the poor nutritive quality of many legume proteins cannot be explained solely on the basis of impaired protein hydrolysis brought about by the presence of enzyme inhibitors in the raw legume seed. Others have pointed out that some mechanisms are involved which are not directly related to the inhibition of normal enzymatic hydrolysis of dietary protein.

A. A. Klose, J. D. Greaves, and H. L. Fevold (*Science* **108,** *88* (*1948*)) found that lima bean fractions with high *in vitro* trypsin-inhibiting activity inhibited the growth of rats on a diet containing acid-hydrolyzed casein as well as on a normal diet.

R. J. Westfall, D. K. Bosshardt, and R. H. Barnes (*Proc. Soc. Exp. Biol. Med.* **68,** *498* (*1948*)) observed that addition of the

active trypsin inhibitor of raw soybeans to a diet containing an enzymatic protein digest caused a decrease in the growth rate of mice, and autoclaving, which inactivated the inhibitor, abolished the harmful effect.

Thus evidence is accumulating that the improved nutritive value of legumes resulting from heat treatment is not consistently related to the presence of proteolytic enzyme-inhibitors.

FOLACIN AND GROWTH OF *LACTOBACILLUS ARABINOSUS* 17-5

The definition of the nutritional indispensability of a substance is occasionally beset with some uncertainty. A case in point is the need for folacin by *Lactobacillus arabinosus* 17-5. One group of investigators (J. C. Lewis, *J. Biol. Chem.* **146,** *441 (1942)*; H. Isbell, *Ibid.* **144,** *567 (1942)*; E. E. Snell and H. K. Mitchell, *Arch. Biochem.* **1,** *93 (1942–43)*) early reported that para-aminobenzoic acid is an essential factor in the metabolism of this organism. However, in another laboratory it was observed that neither para-aminobenzoic acid nor folacin is indispensable for growth of *L. arabinosus* 17-5 (S. Shankman *et al.*, *J. Biol. Chem.* **168,** *23 (1947)*). In a study of para-aminobenzoic acid, para-aminobenzoylglutamic acid, pteroic acid, folacin, and pteroyltriglutamic acid, it was found that all of these compounds supported growth (J. O. Lampen and M. J. Jones, *Ibid.* **166,** *435 (1946)*; **170,** *133 (1947)*; *Nutrition Reviews* **8,** *260, 331 (1950)*). The question obviously needed clarification.

A recent re-examination of this problem has been made using a medium containing a phosphate buffer to prevent deviations of the pH and also less glucose to minimize acid production during the growth of the organism (B. W. Koft, M. G. Sevag, and E. Steers, *J. Biol. Chem.* **185,** *9 (1950)*). At the outset it was found that a solution of folacin whether it be in normal rabbit serum, in phosphate buffer (pH 7.0), or in bacterial medium suffers a marked decomposition in a relatively short time (ten to twenty days). To obviate this difficulty, aliquots were kept frozen at dry-ice temperature, an aliquot being thawed and used for each experiment.

To study further the effect of old solutions of folacin, this factor was incubated in phosphate buffer (pH 7.3) in the dark at 30°C. for ten days. In carefully controlled comparative studies it was found that folacin aged in the medium before inoculation with the test organism, showed a markedly greater growth stimulation than did fresh folacin; mole for mole the fresh vitamin is almost a hundred times less active than aged folacin. This suggests that its decomposition products, rather than folacin itself, account for the growth stimulation. Hence, para-aminobenzoic acid and para-aminobenzoylglutamic acid were compared with fresh and aged folacin with respect to promoting growth of *L. arabinosus* 17-5. The most active are para-aminobenzoic acid and aged folacin followed by para-aminobenzoylglutamic acid. Fresh folacin is the least active under these experimental conditions. It thus appears that folacin *per se* is not a growth stimulant for *L. arabinosus* 17-5; rather it is the decomposition products of this vitamin which promote growth in this organism.

An analogous conclusion may be drawn with respect to the inhibition of growth of *L. arabinosus* 17-5 caused by sulfanilamide (M. G. Sevag, B. W. Koft, and E. Steers, *J. Biol. Chem.* **185,** *17 (1950)*). When the inactivation of sulfanilamide by the metabolic acid produced by this organism is prevented, aged folacin is superior to fresh folacin in antagonizing the growth inhibition by this drug. Again, para-aminobenzoic acid and para-aminobenzoylglutamic acid seem to function more effectively as antagonists to sulfanilamide than does folacin itself.

NOTES

Vitamin P ("Vitamin C_2") and Thyroid Activity

That experimental scurvy in the guinea pig is accompanied by an increase in the weight of the thyroid and various histologic manifestations of thyroid hyperactivity was shown as early as 1915 and has been confirmed many times since (*cf.* M. Gabe and J. L. Parrot, *J. de physiol.* **40,** *63* (*1948*); V. A. Drill, *Physiol. Rev.* **23,** *355* (*1943*)).

P. Crepax (*Arch. Sci. biol.* (*Napoli*) **33,** *298* (*1949*)) has demonstrated that administration of large amounts of ascorbic acid prevented thyroid hyperplasia in guinea pigs receiving daily injections of thyrotropic hormones. Since it has been shown that certain substances with vitamin P activity work in synergism with ascorbic acid in a variety of conditions (H. Cotereau, "Contribution à l'étude des rapports entre l'acide ascorbique et la vitamine C_2 (vitamine P)," *Vol. I, p. 110, Maloine, Paris* (*1947*)), it appeared worth while to study the possible interrelationship between thyroid hormone and vitamin P substances. Using catechin as the vitamin P-active test substance, M. Gabe and J. L. Parrot (*loc. cit.* and *J. de physiol.* **42,** *259* (*1950*)) have been able to demonstrate the following effects:

(1) In the guinea pig, a deficiency of ascorbic acid and vitamin P produces a hyperplasia and signs of hyperactivity of the thyroid which can be eliminated either by administration of large doses of ascorbic acid or by simultaneous administration of small doses of ascorbic acid and catechin.

(2) Injecting normally-fed rats and guinea pigs with an "antivitamin P," obtained by oxidation and polymerization of catechin, causes thyroid hyperplasia and hyperactivity. Large doses of catechin bring the histologic picture back to normal.

(3) Administration of catechin minimizes the effects of injection of thyrotropic hormone on the thyroid of young guinea pigs.

(4) Catechin prevents the hyperplasia of the thyroid caused by thiourea provided small quantities of ascorbic acid are present in the diet. Very large doses of ascorbic acid alone have the same effect. In both cases, histologic examinations indicate that the effect on the thyroid is a direct one and is not mediated through the pituitary. If the diet is deficient in ascorbic acid, administration of catechin has no effect. Here, too, the action of catechin seems to be that of sparing ascorbic acid.

This work brings additional proof of the synergism between vitamin P substances and ascorbic acid, and that vitamin C activity and ascorbic acid content may not always be synonymous terms.

Sensitivity to Vitamin B_{12} Concentrates

One of the difficulties in the treatment of pernicious anemia has been that after prolonged treatment many patients became sensitive to liver extract. Laborious desensitization procedures or intensive oral therapy were not always successful in overcoming these difficulties.

W. C. Young, C. W. Ulrich, and P. J. Fouts (*J. Am. Med. Assn.* **143,** *893* (*1950*)) described a patient with pernicious anemia who became extremely sensitive to liver extract. Desensitization was only temporarily successful. Treatment was changed to a vitamin B_{12} concentrate made from streptomycin broth. This, too, was temporarily successful but eventually the patient developed an alarming reaction after receiving an injection of this material. Therapy was then changed to crystalline vitamin B_{12} made from streptomycin broth. No evidence of sensitivity was observed. Skin sensitivity tests showed no reaction to vitamin B_{12} itself, but did show definite reactions with liver extract and vitamin B_{12} concentrates. Many other clinicians have observed similar cases. Since pure vitamin B_{12} seems to be completely effective in the treatment of pernicious anemia, and since it is readily available commercially, these cases should no longer be a problem.

Nutrition Committee for South and East Asia

The report of the second meeting of the Nutrition Committee for South and East Asia (*FAO Report, Washington, D. C., April 1950*) has now been released and covers both the results obtained in the field of nutrition in the region covered since the previous meeting and recommendations to member governments for further improvements. Participants in the meeting, held under the auspices of the Nutrition Division of FAO were Burma, Ceylon, France, India, Indonesia, Korea, the Philippine Republic, Portugal, Thailand, the United Kingdom, the United States of America, and representatives of the Supreme Commander for the Allied Powers in the Pacific, UNESCO, UNICEF, and WHO. Two subcommittees, one on food composition and one on nutrition education were held simultaneously.

Topics discussed were in line with those examined during the previous meeting, held at Baguio (Philippine Republic) (*FAO Report, Washington, D. C., February 1948*). Frequent reference was made to the publication prepared at that period by the Nutrition Division of FAO ("Nutrition Problems of Rice-Eating Countries in Asia," *FAO Report, Washington, D. C., June 1948*) and to the reports of the International Rice Commission. The main points examined were the following:

(1) Improvement of rice as consumed, which implies obtaining sound nutritional standards for rice, practical methods of estimation of thiamine, improvement of milling technics, wider dissemination of parboiling practices, and if necessary, enrichment. Special attention was paid to recommendations concerning washing and cooking, methods of demonstration of proper processing, and improvement of rice varieties.

(2) Improvement of rice diets, particularly through the use of locally available supplements, like soya bean "milk."

(3) Feeding of vulnerable groups, and full use of skim milk stocks and native foods of high nutritional value.

(4) Feeding of industrial and plantation workers, with the necessity of organizing canteens and community agricultural projects.

(5) Nutrition education and the training of nutrition workers, particular attention being devoted to the full use of existing educational, civic, and medical organizations, visual aids to education, and programs of outside fellowships.

(6) Development of national nutrition organizations, entrusted with coordination of the various branches of government in the field.

(7) Nutrition research, with particular attention given to food analysis, dietary surveys, and mass assessment of nutritional status. The various countries represented were urged to devote a special effort to the testing of the recommendations of the recent international committee on calorie requirements. Finally, the FAO plans for technical assistance to the region of South and East Asia were reviewed and discussed.

Recent Nutrition Books

The Composition of Milks. (Bull. National Res. Council, No. 119, January 1950.) Prepared by Icie G. Macy, Harriet Kelley, and Ralph Sloan for The Food and Nutrition Board. Published by the National Research Council, National Academy of Sciences, Washington 25, D. C. Pp. 64.

Cooperative Determinations of the Amino Acid Content, and of the Nutritive Value of Six Selected Protein Food Sources. Issued by the Bureau of Biological Research, Rutgers University, New Brunswick, N. J. 1950. Pp. 114. Price: $1.50. (Copies of the report may be obtained from Dr. William H. Cole, Rutgers University, New Brunswick, N. J.)

FAO: Calorie Requirements. Report of the Committee on Calorie Requirements. Food and Agriculture Organization of the United Nations, Washington, D. C. June 1950. Pp. 65. Price: $0.75.